Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11239–11243, December 1992
Cell Biology

Identification of a human src homology 2-containing protein-tyrosine-phosphatase: A putative homolog of *Drosophila* corkscrew

(signal transduction/tyrosine phosphorylation/development)

ROBERT M. FREEMAN, JR.*†‡, JORGE PLUTZKY§, AND BENJAMIN G. NEEL†

*Committee on Virology, Harvard University, 260 Longwood Avenue, Boston, MA 02115; §Division of Cardiology, Department of Medicine, The Brigham and Women's Hospital, Boston, MA 02118; and †Molecular Medicine Unit, Beth Israel Hospital, 330 Brookline Avenue, Boston, MA 02215

Communicated by Christopher T. Walsh, August 17, 1992 (received for review July 15, 1992)

ABSTRACT **src homology 2 (SH2) domains direct binding to specific phosphotyrosyl proteins. Recently, SH2-containing protein-tyrosine-phosphatases (PTPs) were identified. Using degenerate oligonucleotides and the PCR, we have cloned a cDNA for an additional PTP, SH-PTP2, which contains two SH2 domains and is expressed ubiquitously. When expressed in *Escherichia coli*, SH-PTP2 displays tyrosine-specific phosphatase activity. Strong sequence similarity between SH-PTP2 and the *Drosophila* gene corkscrew (*csw*) and their similar patterns of expression suggest that SH-PTP2 is the human corkscrew homolog. Sequence comparisons between SH-PTP2, SH-PTP1, corkscrew, and other SH2-containing proteins suggest the existence of a subfamily of SH2 domains found specifically in PTPs, whereas comparison of the PTP domains of the SH2-containing PTPs with other tyrosine phosphatases suggests the existence of a subfamily of PTPs containing SH2 domains. Since corkscrew, a member of the terminal class signal transduction pathway, acts in concert with D-*raf* to positively transduce the signal generated by the receptor tyrosine kinase torso, these findings suggest several mechanisms by which SH-PTP2 may participate in mammalian signal transduction.**

Protein tyrosyl phosphorylation is an important cellular regulatory mechanism. Much work has implicated protein-tyrosine kinases (PTKs) in the control of cell proliferation and differentiation. Many PTKs, when mutated and/or captured by retroviruses, can promote oncogenesis (1–3). In addition, several PTKs are essential for normal development. For example, the *Drosophila* gene torso is essential for proper formation of anterior and posterior structures (4, 5). The *Caenorhabditis elegans let-23* gene regulates vulval development (6) and murine c-*kit* is required for normal hematopoiesis (7, 8).

Much has been learned about PTK signal transduction pathways (2). Many growth factor (GF) receptors are transmembrane PTKs, which autophosphorylate upon ligand addition. Activated GF receptors are linked to downstream cellular events by recruitment of secondary signaling molecules to autophosphorylated receptors; some of these signaling molecules are also substrates for the receptor PTKs. Secondary signaling molecules such as GTPase-activating protein (9), phospholipase C-γ (10, 11), and phosphatidylinositol 3-kinase (12–14) associate with activated receptors through src homology 2 (SH2) domains, conserved stretches of ≈100 amino acids that promote intra- or intermolecular protein–protein interactions via binding to specific phosphorylated tyrosyl residues (15). Since different subsets of phosphotyrosyl proteins bind to different SH2 domains with varying avidity, the specificity of the cellular response to GFs may be largely determined by the strength and spectrum of SH2–phosphotyrosyl interactions (15).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

The steady-state level of protein tyrosyl phosphorylation is also controlled by the opposing action of protein-tyrosine-phosphatases (PTPs). Little is known, however, about the role of PTPs in signal transduction. Molecular cloning has revealed that PTPs can be grouped into two families (16). Transmembrane PTPs, such as CD45 (17) and LAR (18), are thought to be receptors for as yet unidentified ligands. The nontransmembrane PTPs can be grouped into subfamilies based on structural features of their noncatalytic regions, including hydrophobic C-terminal sequences (19–21) in PTP-1B and T-cell PTP, or cytoskeletal protein domains in PTP-Meg (22) and PTP-H1 (23). Whether these structural similarities have functional significance is not known.

Previously, we (24) and others (25–27) identified a nontransmembrane PTP, SH-PTP1 (also known as PTP-1C, HCP, and SHP), which contains two SH2 domains N-terminal to its PTP domain. The presence of SH2 domains in a PTP suggested several possible roles for SH-PTP1 in signal transduction (24–27). Further clues were provided by the recent finding that the *Drosophila* gene corkscrew (*csw*) is also an SH2 domain-containing PTP (28). *csw* functions in the terminal class signal transduction pathway in concert with the l(1) polehole gene product to positively transduce signals generated by torso, a receptor PTK (28). l(1) polehole (D-*raf*) is the counterpart of the mammalian serine/threonine kinase c-*raf* (29). Based on sequence similarity between *csw* and SH-PTP1, Perkins *et al.* (28) suggested that SH-PTP1 might be the mammalian *csw* homolog. However, unlike SH-PTP1, found mainly in hematopoietic cells (24, 25, 27), *csw* is expressed ubiquitously during embryogenesis (28).

We have cloned a second mammalian SH2-containing PTP, SH-PTP2.¶ Comparisons of the SH2 and PTP domains of SH-PTP1, SH-PTP2, and *csw* suggest the existence of a subfamily of SH2 domains found specifically in tyrosine phosphatases and a subfamily of tyrosine phosphatases that contain SH2 domains. Moreover, the striking overall similarity between *csw* and SH-PTP2 and its ubiquitous pattern of expression suggest that SH-PTP2 is the mammalian homolog of *csw*.

MATERIALS AND METHODS

PCR. Degenerate oligonucleotides [sense, AA(A/G)TG(C/T)(C/G)(A/C)X(C/G)A(A/G)TA(C/T)TGGCC; antisense, CCXA(C/T)XCCXGCXGA(A/G)CA(A/G)TGXAC] to conserved catalytic domain sequences of known PTPs were synthesized (24) and used to prime PCRs in which 100 ng of bacteriophage DNA from a λgt11 rat brain cDNA

Abbreviations: SH2, src homology 2; PTK, protein tyrosine kinase; PTP, protein-tyrosine-phosphatase; GF, growth factor; GST, glutathione *S*-transferase.

‡To whom reprint requests should be addressed.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. LO3535).

library (D. Chikaraishi, Tufts University School of Medicine) were used as template. Amplification conditions were as described (24), except that primer annealing was at 37°C for three cycles followed by 50°C for 27 cycles.

Isolation, Sequencing, and Analysis of PTP Clones. PCR fragments of approximately the same size (≈300 base pairs) as that generated using PTP-1B as template were excised and subcloned into pBluescript KS− (Stratagene). PTP clones were identified by a negative selection procedure (see *Results*). One clone, 3B4-15, encoded an unknown PTP. Its insert was radiolabeled (30) and used to screen the rat brain library. A λgt11 human lung cDNA library (Clontech) and a Lambda ZAP II human fetal brain cDNA library (Stratagene) were also screened (see *Results*). Inserts were subcloned into plasmid vectors (for λgt11 clones) or rescued by single-strand helper phage (for Lambda ZAP II clones). Clones homologous to the 3B4-15 fragment (SH-PTP2 clones) included a partial rat brain cDNA, several partial human lung and fetal brain cDNAs, and one human fetal brain cDNA that contains the complete SH-PTP2 coding region. Inserts were sequenced on both strands by the Sanger method (31) or with fluorescent dye technology on a 373A DNA sequencer (Applied Biosystems). DNA and amino acid sequences were analyzed using BLAST (32) or the Genetics Computer Group programs (33).

Bacterial Fusion Protein Expression. A 1.6-kilobase (kb) partial SH-PTP2 cDNA was subcloned into pGEX-3X (Pharmacia) to generate a construct encoding a fusion protein (GST–SH-PTP2) of glutathione *S*-transferase (GST) sequences and SH-PTP2 (amino acids 186–593). Diluted (1:50) overnight cultures were grown to midlogarithmic phase and induced for 4 hr with 0.1 mM isopropyl β-D-thiogalactopyranoside. Bacterial pellets (1 ml of induced culture) were washed with isotonic buffer, lysed in 150 μl of Nonidet P-40 buffer (50 mM Tris·HCl, pH 8.0/150 mM NaCl/1% Nonidet P-40) with protease inhibitors [antipain (1 μg/ml), aprotinin (1 μg/ml), leupeptin (10 μg/ml), pepstatin A (1 μg/ml), phenylmethylsulfonyl fluoride (20 μg/ml)], sonicated (3 min; 0°C), and clarified (10,000 × *g*; 5 min; 4°C). Protein concentrations were determined by BCA assay (Pierce). For affinity purification of GST and GST–SH-PTP2, 80 μl of clarified lysate was adjusted to 2% Triton X-100, incubated (30 min; 22°C) with 30 μl of 50% (vol/vol) glutathione agarose beads (Sigma), and washed three times with isotonic buffer.

PTP Assays. Raytide (Oncogene Science) was phosphorylated as described (24), using 67 μCi (1 Ci = 37 GBq) of [γ-^{32}P]ATP per 10 μg of Raytide. PTP assays were performed on clarified bacterial lysates as described (24). As a percentage of total bacterial protein, substantially more GST than GST–SH-PTP2 is expressed under our conditions.

Northern Blot Analysis. A Northern blot of 2 μg of poly(A)$^+$ RNA from multiple human tissues (Clontech) was hybridized and washed as described (34), using as probe a partial SH-PTP2 cDNA corresponding to nucleotides 599–1881.

RESULTS

Cloning of Human SH-PTP2. Brain contains numerous biochemically distinct tyrosine phosphatase activities (35). For this reason, to identify unknown PTPs, we used degenerate oligonucleotides to two conserved sequences within the phosphatase domain of known PTPs to prime PCRs from rat brain cDNA library DNA. Of the initial 42 clones sequenced, all were derived from the λ phage vector or elongation factor 1α. We radiolabeled these inserts and used them to negatively select the remaining clones by colony hybridization. Of the 48 clones failing to hybridize to these pooled inserts, >50% contained inserts with strong similarity to PTPs. Most were rat homologs of known PTPs, including LRP (36), LAR (18), and HPTPδ (37). One insert, 3B4-15, was obtained (Fig. 1*a*) and used to screen the rat brain library. However, a full-length clone could not be obtained. Northern blotting of RNA from various rat tissues (data not shown) revealed widespread expression, including high levels in lung and brain. We therefore used a partial rat brain cDNA to screen a human lung cDNA library. Again, full-length cDNAs were not obtained. A partial human lung cDNA clone was used to screen a human fetal brain cDNA library. From this library, we obtained several overlapping partial clones as well as one clone containing the complete coding region for SH-PTP2.

Fig. 1*b* shows the nucleotide and deduced amino acid sequence for the coding region of human SH-PTP2. A consensus sequence (AACATGA) for translation initiation (38) at nucleotide 114 is preceded by in-frame stop codons, making it likely that this is the bona fide translational start site. A single open reading frame encoding a 593-amino acid protein (68 kDa) is followed by a 3′ untranslated region with stop codons in all three frames. The remainder of the large 5′ and 3′ untranslated regions has not been characterized.

The predicted amino acid sequence reveals several interesting features. Like other nontransmembrane PTPs, SH-PTP2 contains a single tyrosine phosphatase domain (amino acids 268–525). The cysteine (amino acid 459) previously shown to be essential for catalysis (39) and other residues common to all members of the PTP family are present (Fig. 1*a*, asterisks). N-terminal to the PTP domain are two SH2 domains (amino acids 6–105 and 112–213). Both possess the three invariant residues (15) found in all SH2 domains (Fig. 2, solid dots). Only two of the three conserved basic amino acids believed to participate in interactions with phosphotyrosyl residues (ref. 15; *Discussion*) are present (Fig. 2, daggers). SH-PTP2 has several potential phosphorylation sites for serine/threonine and tyrosine kinases (40, 41). The absence of an apparent nuclear localization signal (42, 43) and the lack of any significant hydrophobic region (44) make it likely that SH-PTP2 is a cytosolic protein, like SH-PTP1 (J.P., U. Lorenz, and B.G.N., unpublished data).

Within the PTP family, human SH-PTP2 is most similar to csw (Figs. 1*a* and 3). Unlike other PTPs, csw contains an insert within its catalytic domain (28); SH-PTP2 lacks such an insert. The PTP domain of SH-PTP2 is 63% identical to csw without its insert. Moreover, the three SH2-containing PTPs are substantially more similar to one another than to any other PTPs (<45%), suggesting the existence of a discrete subfamily of PTPs that contain SH2 domains.

There is also striking sequence similarity between the SH2 domains of the SH2-containing PTPs (Figs. 2 and 3). The SH2 domains of SH-PTP2 are also more similar to csw (≈76%) than to SH-PTP1 (52–63%); they are much less similar to any other SH2-containing protein (<40%). These data establish a discrete subfamily of SH2 domains found primarily in SH2-containing PTPs. Moreover, the similarity between SH-PTP2 and csw extends over the entire sequence of both molecules; overall, the protein sequences of SH-PTP2 and csw are 62% identical. This remarkable similarity between the human SH-PTP2 and *Drosophila csw* genes strongly suggests that SH-PTP2 is the human *csw* homolog.

PTP Activity. To determine whether SH-PTP2 possesses PTP activity, soluble lysates from bacteria expressing GST–SH-PTP2 or GST alone were prepared (see *Materials and Methods*) and equal amounts of protein were assayed by using radiolabeled Raytide. GST–SH-PTP2-expressing lysates showed substantially greater PTP activity than lysates expressing GST alone (Fig. 4). PTP activity was linear over time and proportional to protein concentration. As expected, PTP activity was blocked by Na_3VO_4, a potent tyrosine phosphatase inhibitor (Fig. 4, solid triangle), but was not affected by NaF, a serine phosphatase inhibitor (Fig. 4, open triangle). Similar results were obtained when the respective fusion

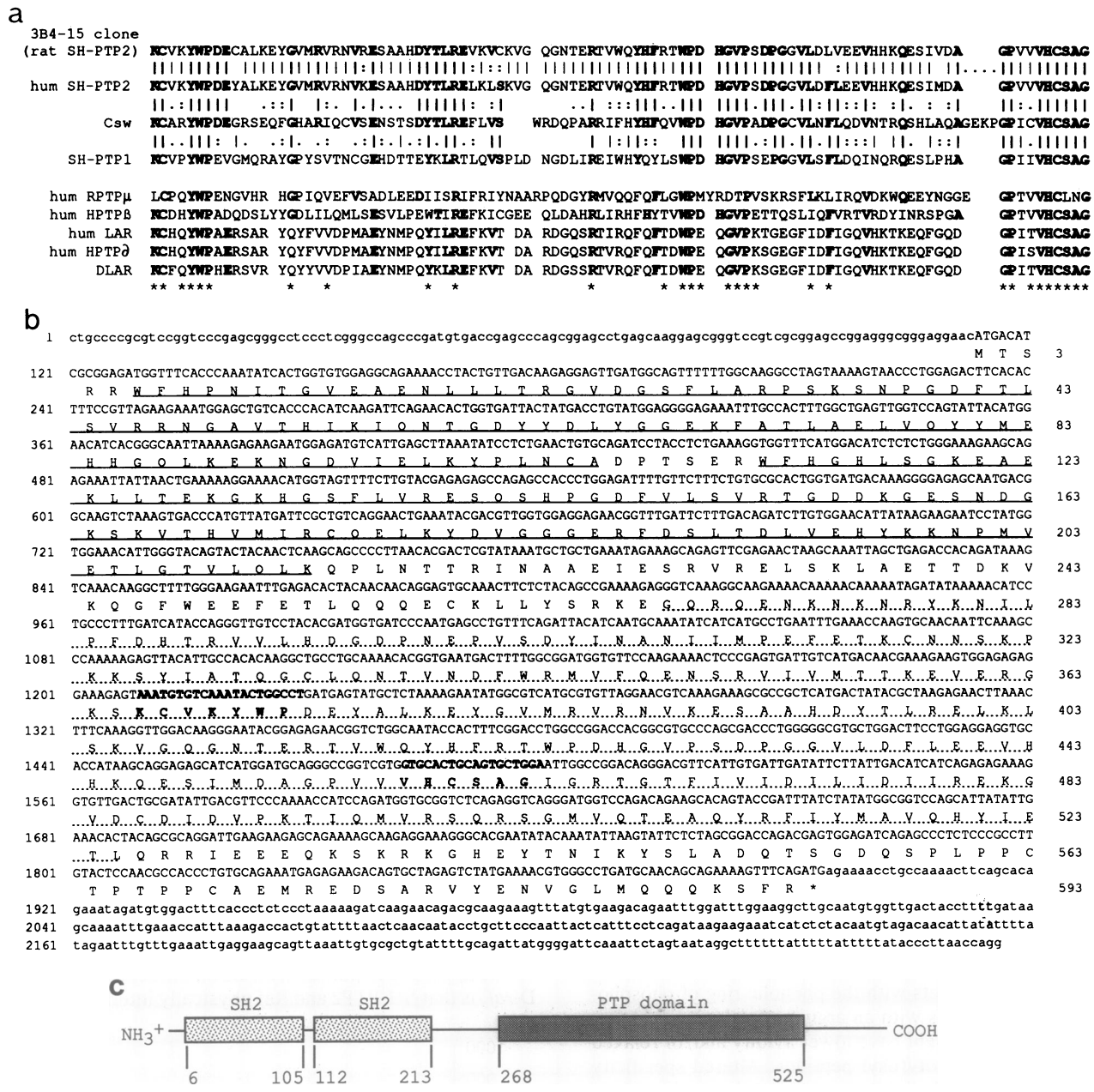

FIG. 1. Sequence and structure of SH-PTP2. (*a*) Sequence comparisons of the phosphatase domains of rat and human SH-PTP2 with other members of the PTP family, as determined by GAP and PILEUP (33). Solid lines indicate identity; conservative substitutions are marked by colons and periods. Residues found in both SH-PTP2 and csw are in boldface type; highly conserved residues found in all PTPs are indicated by asterisks. For proteins with more than one phosphatase domain, the N-terminal domain is shown. Sequence similarity extends throughout the entire PTP domain and is not shown due to space limitations. (*b*) Sequence of human SH-PTP2. Nucleic acid sequence is numbered on the left and predicted amino acid sequence is numbered on the right. The two SH2 domains (solid underline) and the phosphatase domain (dotted underline) are indicated. Positions of the oligonucleotides used to prime the rat brain cDNA library are in boldface. Potential serine/threonine or tyrosine phosphorylation sites (40, 41) include T127, S499, S576, and S591 for protein kinase C; T12, T73, S118, T153, S189, S264, T337, T356, T397, T422, S448, S548, and T553 for casein kinase II; S189, S234, S265, and S576 for S6 kinase; S558, T564, and T566 for cdc2; and Y62, Y63, Y66, Y179, and Y375 for tyrosine kinases. Asterisk denotes stop codon. (*c*) Schematic diagram of predicted protein structure of SH-PTP2. Positions of the two SH2 domains and the PTP domain in the predicted amino acid sequence are indicated.

proteins were affinity purified on glutathione-agarose beads (data not shown).

Gene Structure and Expression. A Northern blot of RNA from various human tissues indicated that SH-PTP2 is expressed as a 6-kb transcript (Fig. 5). Unlike SH-PTP1, which is found almost exclusively in hematopoietic cells, SH-PTP2 is expressed nearly ubiquitously. Notably, SH-PTP2 is expressed in many hematopoietic cells that also express SH-PTP1 (data not shown). Its ubiquitous expression, similar to *csw* (28), is also consistent with the proposal that SH-PTP2 is the mammalian *csw* homolog. Southern blots of human genomic DNA hybridized with the same partial cDNA indicated a complex pattern of bands (data not shown). The large number of bands observed with such a small part of the SH-PTP2 cDNA suggests either that SH-PTP2 is a large gene containing multiple introns or that a family of highly related genes or pseudogenes exists.

DISCUSSION

In this report, we describe the identification of a second mammalian SH2-containing PTP. SH-PTP2 exhibits ty-

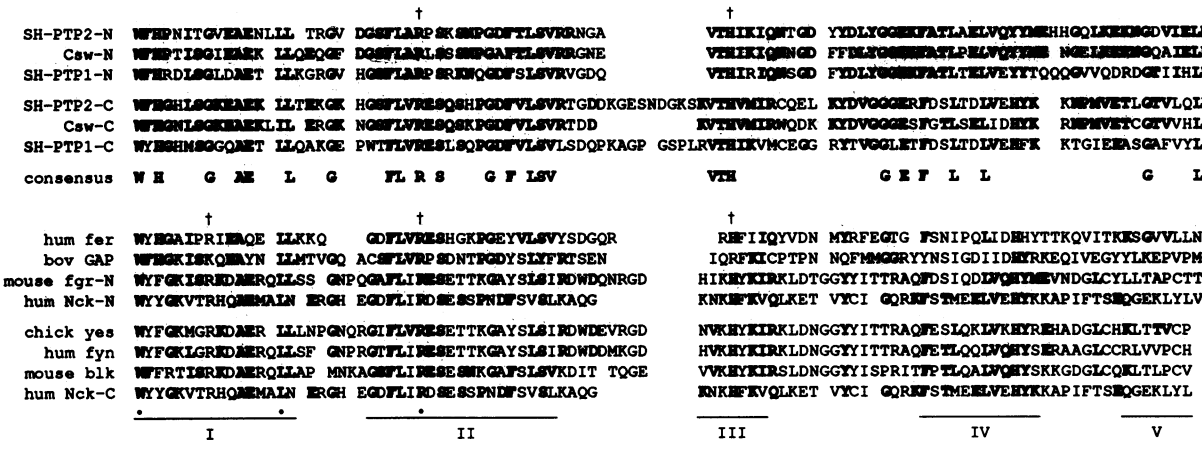

FIG. 2. Sequence comparisons of the two SH2 domains of SH-PTP2, csw, SH-PTP1, and other known SH2 domains. A consensus sequence for the SH2 domains found in SH2-containing PTPs is given. Residues found in both SH-PTP2 and csw are in boldface type. •, Invariant residues in SH2 proteins; †, basic amino acids thought to be involved in phosphotyrosine recognition (15). Proteins with two SH2 domains are suffixed N for N-terminal and C for C-terminal domains. Gapped alignments were made by using PILEUP (33) and are grouped into the conserved SH2 subdomains I–V (15).

rosine-specific phosphatase activity when expressed as a fusion protein in bacteria, is expressed in all tissues examined, and has a complex genomic organization. Comparison of SH-PTP2, SH-PTP1, and *csw* suggests the existence of a subfamily of SH2 domains found specifically in SH2-containing PTPs and a subfamily of PTPs containing SH2 domains. More strikingly, the overall sequence similarity and its pattern of expression suggest that SH-PTP2 is the human homolog of the *Drosophila* gene *csw*.

The specificity and avidity of SH2 domains for phosphotyrosyl residues are dictated by their amino acid sequences (15). Since the SH2 domains of SH-PTPs comprise a distinct subfamily, the SH-PTPs may bind to similar subsets of phosphotyrosyl proteins and the phosphotyrosyl binding partners of the SH-PTPs may be distinct from those bound by other SH2-containing proteins. The first conserved basic amino acid in both SH2 domains of the SH-PTPs is replaced by a glycyl residue. X-ray cocrystallographic studies of the SH2 domain of src (46) indicate that this residue is located on the surface and interacts with the phenolic ring of phosphotyrosine. SH2 domains with an arginine-to-glycine substitution at this position might have lower avidity and/or relaxed specificity for phosphotyrosyl peptides. Altered specificity conceivably could include binding to phosphoseryl or phosphothreonyl residues. However, substitution at the first conserved basic residue is not necessary for relaxed specificity: the SH2 domain of Abl, which does bind to seryl-phosphorylated peptides (47), has an arginine at this position. Moreover, glycyl substitution does not preclude phosphotyrosyl binding, since SH-PTP1 binds to specific phosphotyrosyl proteins (U. Lorenz and B.G.N., unpublished data).

		SH-PTP2	*csw*
PTP domain	SH-PTP2	-	62.5
	SH-PTP1	60.8	57.9
N-terminal SH2 domain	SH-PTP2	-	75.8
	SH-PTP1	63	59.6
C-terminal SH2 domain	SH-PTP2	-	76.1
	SH-PTP1	52	46.1
Overall	SH-PTP2	-	63.2
	SH-PTP1	54.7	50.1

FIG. 3. Sequence similarity between SH2-containing PTPs. SH2 and PTP domains of each protein were compared to one another by using GAP (33), and percentage identity is noted. Comparisons were made to csw without its phosphatase insert.

The similarity between SH-PTP2 and *csw* has interesting implications for the role of SH-PTP2 in signal transduction. Anterior and posterior structures in the *Drosophila* embryo are dependent on the localized activation of the receptor PTK torso, which ultimately activates transcription of the tailless and huckebein transcription factors (48, 49). The torso signal is thought to be conducted via a phosphorylation cascade, at least one component of which is D-*raf* (50). *csw* potentiates the D-*raf* signal (28).

In mammalian cells, the signal generated by many, if not all, GFs appears to be transmitted from receptor PTKs to the nucleus via a pathway(s) dependent on Raf (51, 52). If SH-PTP2 is the mammalian *csw* homolog, it likely acts in proximity to Raf in signal transduction. Some workers have reported a direct interaction between Raf and activated GF receptors and/or that Raf is a substrate of receptor PTKs (53–56). However, this assertion is controversial (reviewed in refs. 51 and 52). Moreover, unlike other proteins that interact with activated GF receptors, Raf lacks SH2 domains.

One appealing hypothesis, based on the genetics of *csw* and D-*raf*, is that SH-PTP2 and Raf physically interact. SH-PTP2

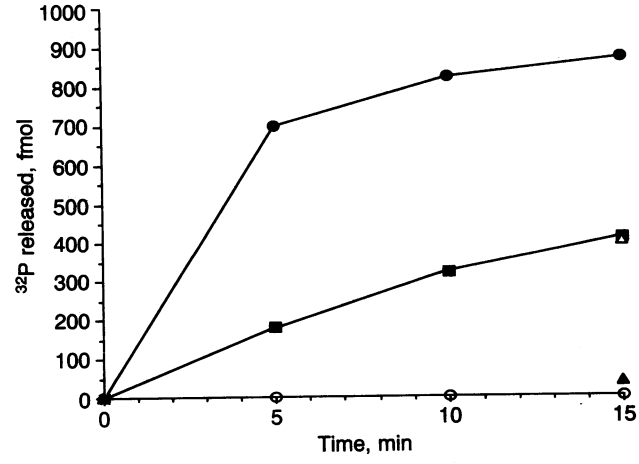

FIG. 4. Time course of dephosphorylation of ^{32}P-labeled Raytide by SH-PTP2. Equal amounts of soluble protein from *E. coli* DH5α expressing GST or GST–SH-PTP2 [10 ng (○) for GST, and 10 ng (●) or 1 ng (■) for GST–SH-PTP2] were incubated with 100 nM ^{32}P-labeled Raytide. At the indicated times, supernatants were assayed for release of ^{32}P by charcoal binding assay (45). GST–SH-PTP2-expressing lysates assayed in the presence of 100 μM Na_3VO_4 (▲) or 50 mM NaF (△) are indicated.

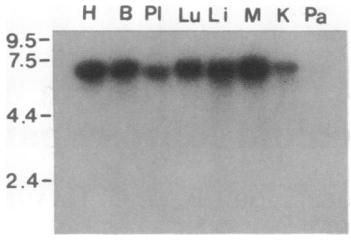

FIG. 5. Expression of SH-PTP2. A Northern blot of 2 μg of poly(A)$^+$ RNA from human tissues was hybridized with an SH-PTP2 partial cDNA, corresponding to nucleotides 599–1881, and washed at high stringency as described (24). H, heart; B, brain; Pl, placenta; Lu, lung; Li, liver; M, skeletal muscle; K, kidney; Pa, pancreas. Numbers on left are kb.

could then act, at least in part, to bring Raf to activated receptors. This might account for the variability seen in Raf tyrosine phosphorylation and receptor association—slight changes in conditions could lead to variable dephosphorylation of Raf by SH-PTP2 during extraction. SH-PTP2 might simultaneously function to dephosphorylate the activated receptor, thus terminating the GF signal. Alternatively, there could be intervening signaling molecules between SH-PTP2 and Raf. Genetic interaction between *csw* and D-*raf* (28) could also imply that SH-PTP2 is a substrate for Raf and/or vice versa.

Any model proposing an SH-PTP2–Raf interaction relies on the proposition that SH-PTP2 is the mammalian *csw* homolog. Despite their striking similarity, SH-PTP2 does differ from *csw* in its lack of an insert in its phosphatase domain. Although it may have a regulatory role (28), the function of the *csw* insert is unknown. Since it has not been determined whether *csw* has PTP activity, it is difficult to predict what type of regulation the insert might exert. Several *csw* transcripts exist, and their relative abundance varies during development (28). The precise genetic content of these transcripts has not been defined. It will be interesting to determine whether all *csw* isoforms have a phosphatase insert. Similarly, it will be important to determine whether insert-containing isoforms of SH-PTP2 exist. Ultimately, proof that SH-PTP2 is the human *csw* homolog will depend on the ability of SH-PTP2 expression constructs to rescue *csw* null mutations. Even if SH-PTP2 is not the true *csw* homolog, the sequence features of the SH2-containing PTPs allow definition of a consensus sequence for SH2 domains associated with PTPs (Fig. 2) and suggest straightforward PCR approaches toward the isolation of other SH2-containing PTPs.

Note Added in Proof. Recent studies in our laboratory indicate that SH-PTP2 binds directly to several activated growth factor receptors via its SH2 domains.

We thank Dr. D. Chikaraishi for the rat brain cDNA library and Drs. L. Perkins (Harvard Medical School) and U. Lorenz for suggestions on the manuscript. This work was supported by grants to B.G.N. from the National Institute of Health (CA49152), the William F. Milton Fund, and the Harvard Medical School/Hoffmann–La Roche Institute for Chemistry and Medicine.

1. Bishop, J. M. (1991) *Cell* **64**, 235–248.
2. Cantley, L., Auger, K., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R. & Soltoff, S. (1991) *Cell* **64**, 281–302.
3. Hunter, T. (1991) *Cell* **64**, 249–270.
4. Casanova, J. & Struhl, G. (1989) *Genes Dev.* **3**, 2025–2038.
5. Sprenger, F., Stevens, L. M. & Nusslein-Volhard, C. (1989) *Nature (London)* **338**, 478–483.
6. Horvitz, H. & Sternberg, P. (1991) *Nature (London)* **351**, 535–541.
7. Geissler, E. N., Ryan, M. A. & Housman, D. E. (1988) *Cell* **55**, 185–192.
8. Chabot, B., Stephenson, D. A., Chapman, V. M., Besmer, P. & Bernstein, A. (1988) *Nature (London)* **335**, 88–89.
9. Kaplan, D. R., Morrison, D. K., Wong, G., McCormick, F. & Williams, L. T. (1990) *Cell* **61**, 125–133.
10. Margolis, B., Rhee, S. G., Felder, S., Mervic, M., Lyall, R., Levitzki, A., Ullrich, A., Zilberstein, A. & Schlessinger, J. (1989) *Cell* **57**, 1101–1107.
11. Meisenhelder, J., Suh, P.-G., Rhee, S. G. & Hunter, T. (1989) *Cell* **57**, 1109–1122.
12. Otsu, M., Hiles, I., Gout, I., Fry, M. J., Ruiz-Larrea, F., Panayotou, G., Thompson, A., Dhand, R., Hsuan, J., Totty, N., Smith, A. D., Morgan, S. J., Courtneidge, S. A., Parker, P. J. & Waterfield, M. D. (1991) *Cell* **65**, 91–104.
13. Skolnik, E. Y., Margolis, B., Mohammadi, M., Lowenstein, E., Fischer, R., Drepps, A., Ullrich, A. & Schlessinger, J. (1991) *Cell* **65**, 83–90.
14. Escobedo, J. A., Navankasattusas, S., Kavanaugh, W. M., Milfay, D., Fried, V. A. & Willians, L. T. (1991) *Cell* **65**, 75–82.
15. Koch, C., Anderson, D., Moran, M., Ellis, C. & Pawson, T. (1991) *Science* **252**, 668–674.
16. Fischer, E., Charbonneau, H. & Tonks, N. (1991) *Science* **253**, 401–406.
17. Charbonneau, H., Tonks, N. K., Walsh, K. A. & Fischer, E. H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7182–7186.
18. Streuli, M., Krueger, N. X., Hall, L. R., Schlossman, S. F. & Saito, H. (1988) *J. Exp. Med.* **168**, 1523–1530.
19. Cool, D. E., Tonks, N. K., Charbonneau, H., Walsh, K. A., Fischer, E. H. & Krebs, E. G. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5257–5261.
20. Chernoff, J., Schievella, A. R., Jost, C. A., Erikson, R. L. & Neel, B. G. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2735–2739.
21. Brown-Shimer, S., Johnson, K. A., Lawrence, J. B., Johnson, C., Bruskin, A., Green, N. R. & Hill, D. E. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 5148–5152.
22. Gu, M., York, J. D., Warshawsky, I. & Majerus, P. W. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5867–5871.
23. Yang, Q. & Tonks, N. K. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5949–5953.
24. Plutzky, J., Neel, B. & Rosenberg, R. (1991) *Proc. Natl. Acad. Sci. USA* **89**, 1123–1127.
25. Yi, T., Cleveland, J. L. & Ihle, J. N. (1992) *Mol. Cell. Biol.* **12**, 836–846.
26. Shen, S.-H., Bastien, L., Posner, B. I. & Chrétien, P. (1991) *Nature (London)* **352**, 736–739.
27. Matthews, R. J., Bowne, D. B., Flores, E. & Thomas, M. L. (1992) *Mol. Cell. Biol.* **12**, 2396–2405.
28. Perkins, L. A., Larsen, I. & Perrimon, N. (1992) *Cell* **70**, 225–236.
29. Ambrosio, L., Mahowald, A. & Perrimon, N. (1989) *Nature (London)* **342**, 288–290.
30. Feinberg, A. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13.
31. Sanger, F., Nicklen, S. & Coulson, A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
32. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215**, 403–410.
33. Devereux, J., Haeberli, M. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387–395.
34. Gebert, J. F., Moghal, N., Frangioni, J. V., Sugarbaker, D. J. & Neel, B. G. (1991) *Oncogene* **6**, 1859–1868.
35. Jones, S. W., Erikson, R. L., Ingebritsen, V. M. & Ingebritsen, T. S. (1989) *J. Biol. Chem.* **264**, 7747–7753.
36. Matthews, R. J., Cahir, E. D. & Thomas, M. L. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4444–4448.
37. Krueger, N. X., Streuli, M. & Saito, H. (1990) *EMBO J.* **9**, 3241–3252.
38. Kozak, M. (1986) *Cell* **44**, 283–292.
39. Streuli, M., Krueger, N. X., Tsai, A. Y. M. & Saito, H. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 8698–8702.
40. Kennelly, P. J. & Krebs, E. G. (1991) *J. Biol. Chem.* **266**, 15555–15558.
41. Kemp, B. E. & Pearson, R. B. (1990) *Trends Biochem. Sci.* **15**, 342–346.
42. Newport, J. & Forbes, D. J. (1987) *Annu. Rev. Biochem.* **56**, 535–565.
43. Dingwall, C. & Laskey, R. A. (1986) *Annu. Rev. Cell Biol.* **2**, 367–390.
44. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157**, 105–132.
45. Streuli, M., Kreuger, N. X., Thai, T., Tang, M. & Saito, H. (1990) *EMBO J.* **9**, 2399–2407.
46. Waksman, G., Kominos, D., Robertson, S. C., Pant, N., Baltimore, D., Birge, R. B., Cowburn, D., Hanafusa, H., Mayer, B. J., Overduin, M., Resh, M. D., Rios, C. B., Silverman, L. & Kuriyan, J. (1992) *Nature (London)* **358**, 646–653.
47. Pendergast, A. M., Muller, A. J., Havlik, M. H., Maru, Y. & Witte, O. N. (1991) *Cell* **66**, 161–171.
48. Bronner, G. & Jackle, H. (1991) *Mech. Dev.* **35**, 205–211.
49. Pignoni, F., Baldarelli, R. M., Steingrimsson, E., Dias, R. J., Patapoutian, A., Merrian, J. R. & Lengyel, J. A. (1990) *Cell* **62**, 151–163.
50. St. Johnston, D. & Nusslein-Volhard, C. (1992) *Cell* **68**, 201–219.
51. Rapp, U. R. (1991) *Oncogene* **6**, 495–500.
52. Li, P., Wood, K., Mamon, H., Haser, W. & Roberts, T. (1991) *Cell* **64**, 479–482.
53. App, H., Hazan, R., Zilberstein, A., Ullrich, A., Schlessinger, J. & Rapp, U. R. (1991) *Mol. Cell. Biol.* **11**, 913–919.
54. Baccarini, M., Sabatini, D. M., App, H., Rapp, U. R. & Stanley, E. R. (1990) *EMBO J.* **9**, 3649–3657.
55. Morrison, D. K., Kaplan, D. R., Rapp, R. & Roberts, T. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8855–8859.
56. Morrison, D. K., Kaplan, D. R., Escobedo, J. A., Rapp, U. R., Roberts, T. M. & Williams, L. T. (1989) *Cell* **58**, 648–657.